in trial 1 and 6% in trial 2, and total 100-day treatment-related mortality was 13% in trial 1 and 6% in trial 2. The incidence of chronic GVHD in the 2 trials was 49% and 44%, respectively. At 2 years, the relapse-free and overall survival estimates for trial 1 are 46% and 48%, respectively (median follow-up, 27 months). One year relapse-free and overall survival estimates for trial 2 are 71% and 75%, respectively (median follow-up, 11 months). Overall survival at 2 years is 72%. **Conclusions:** Sirolimus, when added to tacrolimus after allogeneic stem cell transplantation, is effective as GVHD prophylaxis. Engraftment is prompt, and transplant-related morbidity and mortality is reduced. Survival estimates are excellent due to reduced GVHD and transplant-related toxicity. Sirolimus is worthy of broader study in allogeneic transplantation.

10

SIROLIMUS AND THROMBOTIC MICROANGIOPATHY AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Sirolimus (SRL) is a novel immunosuppressive agent that can reduce GVHD and minimize morbidity after allogeneic stem cell transplantation (SCT). Thrombotic microangiopathy (TMA) is characterized by microangiopathic hemolysis, thrombocytopenia, and renal dysfunction and is associated with calcineurin inhibitors (CIs). To determine whether SRL use potentiates the effects of CIs on TMA incidence and risk factors, we performed a retrospective cohort analysis of subjects who underwent SCT between 1997 and 2003. **Methods:** Subjects who received a SRL-containing GVHD prophylaxis regimen were compared with a cohort who received a non-SRL regimen. All subjects received CIs. Diagnosis of TMA required the simultaneous occurrence of (1) creatinine elevation $>$ 2 mg/dl or $>$ 50% above baseline, (2) schistocytosis, (3) elevated LDH, and (4) no evidence of DIC. **Results:** The 111 patients who received SRL were compared with 216 patients who received no SRL. The 2 groups of patients were balanced for demographic parameters; however, more patients in the SRL group received PBSC transplants (50.5 vs 18.1%; $P < .01$) and had unrelated donors (58.6 vs 42.6%, $P < .01$). The incidence of TMA was higher in the SRL group than in the non-SRL group (10.8% vs 4.2%; OR = 2.57, $P = .03$). SRL patients developed TMA earlier than did non-SRL patients (median, 25 days vs 58 days; $P = .04$). At TMA diagnosis, median blood levels of immunosuppressive medications were all within their respective therapeutic ranges. In a multivariable logistic regression model, only the use of SRL (adjusted exact OR = 3.49, $P = .02$) and grade II-IV acute GVHD (adjusted exact OR = 6.60, $P = .0002$) predicted the occurrence of TMA. Treatment of TMA consisted of discontinuation or dose adjustment of CIs and SRL. Two subjects in each group required temporary hemodialysis, and 3 subjects (1 SRL, 2 non-SRL) underwent plasmapheresis. A total of 78% of surviving SRL-treated subjects regained normal renal function. No subject had a TMA recurrence if SRL was reintroduced. CIs were never reintroduced. Overall survival after TMA diagnosis was better for SRL patients than for non-SRL patients (58.3% vs 11.1%; log rank $P = .02$). **Conclusion:** SRL is associated with an increased risk of TMA after SCT and may act by potentiating the effects of CIs. TMA associated with SRL appears to be reversible and does not affect overall survival after SCT. A careful monitoring strategy for TMA should be used as part of a SRL-containing GVHD prophylaxis regimen.

11

PALIFERMIN (A RHUKGF MOLECULE) IS SAFE AND WELL TOLERATED IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES (HM) UNDERGOING HIGH-DOSE CHEMORADIOTHERAPY (HD-CRT) FOLLOWED BY ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

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The major limitation of allogeneic HSCT is graft-versus-host-disease (GVHD), a complication where the skin, gastrointestinal (GI) tract, and liver are damaged by cytokine effectors. Palifermin has a growth-enhancing differentiation and cytoprotective effect in the oral and GI mucosa and may be useful as a GVHD prophylaxis. Previously, we demonstrated that palifermin significantly reduced the incidence, duration, and severity of oral mucositis (OM) in the autologous HSCT setting. Two animal studies demonstrated palifermin could prevent mucositis and GVHD after CRT. In this investigator-initiated, randomized, double-blind, placebo-controlled, dose-escalation trial, the safety of palifermin administration to HM patients undergoing HD-CRT following by allogeneic HSCT was evaluated. **Methods:** Patients were entered into 1 of 3 sequentially-enrolled dose/schedule cohorts involving administration of study drug (placebo or palifermin) on days −11, −10, −9, 0, 1, and 2 relative to HSCT (day 0). Three additional doses were given to each sequential cohort: stage 1 (3 pretreatment/3 posttreatment), stage 2 (3 pretreatment/6 posttreatment), or stage 3 (3 pretreatment/9 posttreatment). Adverse events (AEs) were collected continuously. Patients were followed for safety for 100 days and for survival/relapse for 1 year. **Results:** A total of 100 patients (31 placebo, 69 palifermin [8 receiving 40 μg/kg/day and 61 receiving 60 μg/kg/day]) were randomized to stage 1, 2, or 3. Time to engraftment for ANC and platelets was similar between treatment groups. No significant differences in the incidence or severity of GVHD, survival, or relapse rates were observed between treatment groups. Overall, 20 patients (2 placebo, 18 palifermin) discontinued the study. Of these, 6 patients (2 placebo, 4 palifermin) experienced a total of 11 dose-limiting toxicities (DLTs). Discontinuations and DLTs were most common in patients who received at least 6 doses of study drug after engraftment (day 0). Eighteen patients (5 placebo, 13 palifermin) died before day 100, due primarily to treatment complications. Palifermin appeared to have a beneficial effect on OM, but no effect was observed on diarrhea. **Conclusion:** Palifermin administration in the allogeneic treatment setting was safe and well tolerated and did not have any negative effects on engraftment, GVHD, or survival rates.

12

ADOPTIVE TRANSFER OF IN VITRO-GENERATED T CELL PRECURSORS ENHANCES POSTTRANSPLANT DONOR T CELL RECONSTITUTION IN RECIPIENTS OF AN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Recent studies have shown that murine T cells and their precursors can be generated from hematopoietic stem cells (HSC) in vitro using a novel OP9-DL1 co-culture system consisting of OP9 bone marrow stromal cells expressing Delta-like 1 Notch ligand (which binds to the Notch 1 receptor) and growth factors (interleukin 7 and fms-like tyrosine kinase-3 ligand). In this study we determined the effects of ex vivo-generated T cell precursors on T cell reconstitution after allo HSCT. We selected Lin-, Sca-1hi, and c-kithi HSC from donor bone marrow and cultured these on a monolayer of OP9-DL1 cells in the presence of growth factors. These HSC expanded 850-5000-fold and consisted of 95% CD4-CD8 double-negative (DN) T cell precursors after 16–28 days of culture. We infused these cells (8 × 106) with T-cell- depleted (TCD) BM (5 × 106) into allogeneic recipients. Control mice received TCD BM only. To determine the effects on T cell reconstitution after allo HSCT we analyzed thymi and spleens at days 14 and 29 after transplant by flow cytometry. Progeny of OP9-DL1 T cell precursors was found on days 14 and 29 in both thymus and spleen, including 40% (± 20%) of thymocytes and 25% (± 9%) of splenic T cells on day 14 posttransplant and 3.6% (± 2.4%) of thymocytes and 33% (± 7%) of splenic T cells on day 29 posttransplant. The thymic cellularity was increased up to 50% on day 14 posttransplant in the OP9-DL1 HSCT group compared to the control group. Splenic T cell numbers were not increased in OP9-DL1 recipients; however, donor T cell chimerism was enhanced significantly 88.5% vs 65%).